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Training Dogs for Awake, Unrestrained Functional Magnetic Resonance Imaging

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Dr. Philip Steindel, Review Editor
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617.674.1888

Dear Dr. Steindel,

Enclosed is a revision of the invited manuscript, JoVE60192 “Training Dogs for Awake, Unrestrained Functional Magnetic Resonance Imaging” by Strassberg, Waggoner, Deshpande, and Katz which we would like you to consider as an article for the Journal of Visualized Experiments. As requested we have used track changes within the manuscript to identify all of the edits.

Thank you in advance for your efforts and we look forward to hearing from you soon.

Sincerely yours,

Jeffrey S. Katz, Ph.D.
Professor
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TITLE:
Training Dogs for Awake, Unrestrained Functional Magnetic Resonance Imaging

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SUMMARY:
Magnetic resonance imaging (MRI) on unrestrained awake dogs is a new method with several advantages over imaging with physical or chemical restraint. This protocol introduces a cost-effective training method that minimizes training in the MRI environment, which can be expensive, and maximizes the subject pool available for canine functional MRI.

ABSTRACT:
We present a canine functional Magnetic Resonance Imaging (fMRI) training protocol that can be done in a cost-effective manner, with high-energy dogs, for acquisition of functional and structural data. This method of training dogs for awake, unrestrained fMRI employs a generalization procedure of stationing in several dissimilar locations to facilitate transfer of the stationing behavior to the real MRI scan environment; it does so without the need for extensive training time in the MRI scan environment, which can be expensive. Further, this method splits the training of a stationing (i.e., chin rest) behavior from desensitization to the MRI environment (i.e., 100+ decibel scan audio), the latter accomplished during dedicated Auditory Exposure conditioning sessions. The complete training and testing protocol required 14 hours and resulted

in immediate transfer to novel locations. We also present examples of canine fMRI data that have been acquired from visual face processing and olfactory discrimination paradigms.

INTRODUCTION:

Magnetic resonance imaging (MRI) conducted on unrestrained awake dogs is a new method, creating a fresh way to examine function and structure in the dog brain. The first published accounts of MR image acquisition from unrestrained awake dogs were published in 2009 (structural) and 2012 (functional)[1,2]. There are several advantages of functional magnetic resonance imaging (fMRI) for studying brain function in unrestrained awake dogs. First, the data collection is similar to that of humans, and therefore more readily generalizable across species[3]. Second, there is no need for anesthesia, eliminating any undesirable aftereffects. Third, brain activity is altered by anesthesia and hence cognitive function can be better assessed without anesthesia[4]. Fourth, while fluid/food deprivation and physical restraint allow researchers to probe nonsedated animals (e.g., rodent, avian, and primate models), those animals can be in very different cognitive states from their non-deprived and unrestrained counterparts[3].

At the moment, there are five laboratories around the world that are scanning awake dogs (Atlanta, USA; Auburn, USA; Budapest, Hungary; Querétaro, Mexico; Vienna, Austria), and there is no standardized method for training dogs to willfully undergo an MRI scan[5-7]. All the training methods share the common goal of training dogs to remain still for extended periods of time, which is necessary for quality brain scans. While all methods work via the principles of reinforcement learning, how exactly it is implemented varies, and we do not yet know the impact of this variance on the results. Therefore, if a proposed training method is accepted and comes to be widely used, it may reduce some amount of undesirable variance in the data. In this article, we focus on the training method for stationing in the MRI scanner. MRI scanning is expensive, and the proposed method we developed has the purpose of being cost-effective and thus generalizable to trainers around the world without regular access to an MRI scanner for training.

The method consists of two major components: training and testing. Training consists of two phases. Phase one is training the dog to chin target (i.e., station) in an open environment and phase two is training the dog to station in a mock MRI. Desensitization to the MRI occurs throughout the training phases, during separate, dedicated Auditory Exposure sessions. Testing consists of stationing in a portable mock MRI, in five different testing locations. The utility of this testing phase is to generalize the stationing behavior, facilitating transfer to the real MRI environment. The overall protocol is summarized in **Figure 1**.

[Place **Figure 1** here]

Depending on the phase, training and testing takes 25 to 75 minutes per week, per dog: one 10-minute Auditory Exposure session and two or more 5 to 30-minute Stationing sessions. This protocol can be completed in 25 weeks. During transfer testing, dogs execute several bouts of a 5-minute motionless down/stay and chin rest in a portable mock MRI (bore, radiofrequency coil, 90+ dB audio, ear padding) in five dissimilar locations. Transfer sessions occur once per week for 30–60 minutes, over five consecutive weeks. During MRI testing, dogs execute several bouts of the final stationing behavior during a 60-minute session of structural and functional data acquisition in a real MRI scanner.

Throughout training and testing, a chin rest is the behavior of focus. A chin rest is the dog touching his chin to an object's surface following some cue to target (i.e., rest his chin) to that surface. That cue to target can be physical (e.g., gesture, lure), verbal (e.g., spoken word “rest”), or an object (e.g., access to the chin rest itself). Fluent performance of the chin targeting behavior is critical to limiting head motion. In this protocol, the chin rest behavior is conditioned, maintained, and generalized to occur in multiple contexts (different rest apparatuses, in multiple locations) with increasing target duration (up to five minutes). Additionally, the trainer conditions and maintains strong performance of behaviors down and stay, as well as good stimulus control over the release cue “Okay,” the conditioned reinforcer and behavioral event marker “click,” and the Keep Going Signal (KGS) “good”[8]. Over the course of the protocol, multiple stimuli and apparatuses are introduced at specific stages and for specific intervals. These materials are easily and inexpensively procured. For full details, see the **Table of Materials**.

PROTOCOL:

Ethical approval for these methods was obtained from the Auburn University Institutional Animal Care and Use Committee and all methods were performed in accordance with their guidelines and regulations. For auditory exposure, progression through the sessions is based on week number. For stationing sessions, a specific session-specified performance criterion (e.g., at least eleven-second duration of chin targeting), must be met before the trainer may advance the dog to the next session in that training phase. Otherwise, that step is repeated.

1. Auditory Exposure sessions

NOTE: These sessions constitute passive exposure and active classical counterconditioning of a positive Conditioned Emotional Response (CER) to MRI scanner noise; the scanner noise is established as a stimulus predicting the access to toy play or food rewards. Exposure sessions occur once per week for approximately 10 min.

1.1. Passive Exposure (PE) sessions are ambient, 40–70 decibel (dB) exposure to MRI scanner noises. Transport the dog to a familiar exercise area and allow the dog to walk around while audio playback is quietly audible at the session-specified volume through a portable Bluetooth speaker (see **Table of Materials**). Conduct three PE sessions of 10 min playback of scanner noise, once per week for three weeks (PE_1 40–60 dB, PE_2 65 dB, PE_3 70 dB).

1.2. Active Exposure (AE) sessions utilize a standard classical conditioning paradigm and are conducted after the three PE sessions. Transport the dog to a familiar indoor training room and run through ten trials of the short-delay classical conditioning procedure (see **Figure 2**).

1.2.1. Play scan audio at the session-specified volume for 10 s.

1.2.2. After 10 s have elapsed, engage in 20 s of toy-play (or continuous food reward) with the dog while the scanner noise is still audible.

1.2.3. After 20 s of play, retrieve the toy from the dog and pause the noise. Wait with the dog (in silence, dog without toy/food) for 10 s.

1.2.4. After this delay, start the next trial. Conduct ten trials per session.

1.2.5. Volume is incrementally increased over sessions. Conduct an AE session once per week for twelve weeks (AE_1 45 dB, AE_2 50 dB, AE_3 55 dB, AE_4 60 dB, AE_5 65 dB, AE_6 70 dB, AE_7 75 dB, AE_8 80 dB, AE_9 85 dB, AE_{10} 90 dB, AE_{11} 100 dB, AE_{12} > 100 dB).

[Place **Figure 2** here]

NOTE: Collect the following audio: MRI scanner baseline, shim, localizer (scout), MPRAGE, GRE Field, EPI, Multiband EPI, DTI, and RESOLVE DTI, using, for example, a smartphone's audio recording app through the open door of a 3T MRI suite during phantom scans. Determine volume level of audio playback during training sessions via a decibel meter phone app.

2. Stationing sessions

NOTE: These sessions are divided into two phases: Open environment and Mock MRI. After the chin-to-object target is learned, durations are increased on a percentile schedule of 10% increases. As new elements and pieces of equipment are added into the training context, certain criteria of the behavior (e.g., duration) are temporarily relaxed:
1) In the stationing sessions, the trainer trains a nose-touch behavior to a folded towel and then a chin rest on a folded towel. That chin rest behavior is generalized to occur in a foam chin rest and gradually built to a 5 min bout duration.
2) Simultaneously, robust down and stay behaviors are built and maintained.
3) Those behaviors are then conditioned to occur in an enclosed space (i.e., tunnel) and at a 3' elevation.
4) The dog is then acclimated to the head enclosure (mock human extremity RF coil).
5) Ear padding is introduced, and scan audio is (re)introduced in the context of the stationing behavior.
The dog will ultimately be able to perform a robust chin rest with head and body enclosed at a 3' elevation, with ear padding and scan audio playing at 90 + decibels (dB), for at least 5 min bouts.
On reinforcement – some dogs are inherently more motivated by food, whereas others are more motivated by play or praise[9]. In "click-then-treat" (C/T), the T does not necessarily mean food treats, rather it refers to the reward procedure, whatever that may be for that particular dog at that particular stage in its training. Although food rewards lend themselves to higher rates and stiller repetitions of behavior, whatever the dog prefers can be used initially, even if it is high-motion play (e.g., ball, tug). As the chin target behavior becomes more resilient against distraction and duration, transition to using food rewards. Eventually, toy play can be saved for

long-duration or chained bouts of chin rest performance.

2.1. **Phase 1 – Open environment**

2.1.1. *Charge the clicker.* Build an association between the 'tic-toc' of the clicker and the dog's primary reward (e.g., food) while capturing attention. Conduct rapid repetitions of C/T events for attention (body orientation towards and/or eye contact); toss the treats to reset the dog's position. Conduct this session once.

NOTE: This session usually takes no more than 3 min. It can be longer or repeated if the trainer is not seeing signs that the clicker is being established as a conditioned reinforcer—orientation towards the trainer and emission of reward-seeking behaviors upon hearing the click are good indications that the clicker is becoming a meaningful signal to the dog.

2.1.2. *Capture chin target to towel.* With the dog standing, sitting, or in a down, C/T for looking at, then investigating (i.e., sniffing) the towel. Once that is occurring reliably, C/T for any nose-to-towel, and then chin-to-towel contact. Build towel contact duration to 2 s.

2.1.2.1. Repeat this session until the dog will chin target for 2 s; each session should last approximately 5 min.

NOTE: If a dog is struggling with this step, the trainer may (a) rub a treat on the towel to get the behavior started via a food odor lure and/or (b) teach the dog a nose target (nose-to-palm), then a chin target (chin-to-palm), and then cue for a chin target over the towel.

2.1.3. *Chin-to-towel target with short duration and addition of cue*. With the dog standing, sitting, or in a down, C/T for 1–2 s of chin contact to the towel. Say "rest" while the dog is touching or about to touch the towel. After many repetitions of 1–2 s bouts, C/T after 3, then 4, then 5, then 7 s.

2.1.3.1. Repeat this session until the dog will chin target for 7 s; each session should last approximately 5 min.

NOTE: Vary the bout lengths of each chin rest so that the next repetition is not always longer than the previous repetition (i.e., 1", 1", 3", 1", 5", 2", 6", 4", 1", 2", 7", instead of, 1", 1", 1", 1", 2", 2", 3", 4", 5", 6", 7").

2.1.4. *Chin rest on towel in a down and addition of distraction.* With the dog in a down, cue "rest" and C/T for 1–5 s of chin-to-towel contact. Gradually add visual and acoustic distractions in the form of the trainer moving her hands and feet (e.g., knock on ground, wiggle fingers, shuffle foot, etc.). Build chin-to-towel contact duration to 11 s. Increments can be 1"–5", 6"–7", 8", 9–10", 11"+.

2.1.4.1. Repeat this session until the dog will chin target for 11 s; each session should last approximately 5 min.

2.1.5. *Chin rest on towel with distance.* With the dog in a down beside a folded towel or stack of folded towels, cue "rest" and C/T for 1–3 s of chin-to-towel contact. Cue the behavior from progressively farther away (i.e., sitting on ground, kneeling, standing). Build chin-to-towel contact duration to 16 s. Increments can be 1"–3", 4"–8", 9"–11", 12"–14", and then 16"+.

2.1.5.1. Repeat this session until the dog will chin target for at least 16 s; each session should last between 5 and 10 min.

2.1.6. *Chin rest on towel with increasing duration and distance.* With the dog in a down beside a folded towel or stack of folded towels, cue "rest" and C/T for 1–11 s of chin contact. Build chin-to-towel contact duration to 26 s. Increments can be 1"–11", 12"–16", 17"–19", 21"–23", 26"+.

2.1.6.1. Repeat this session until the dog will chin target for at least 26 s; each session should last between 5 and 10 min.

2.1.7. *Introduce foam chin rest.* C/T for any investigation (i.e., sniffing, close proximity, orientation towards) of the foam chin rest apparatus. After several reinforced investigations of the apparatus, cue "rest" and C/T for 1–10 s of chin contact. Build duration to 40 s. Increments can be chin rest for 1"–10", 11"–21", 23"–31", 40"+.

2.1.7.1. Repeat this session until the dog will chin target for at least 40 s; each session should last between 5 and 15 min.

NOTE: Construct the rolled foam chin rest apparatus to fit each individual dog's muzzle; for a Labrador-sized dog, make a foam chin rest measuring 6" wide with a 4" wide by 2.5" deep notch cut out of the middle. This material is safe to take into the MRI environment.

2.1.8. *Chin rest in foam chin rest with increasing distraction and duration.* With the dog in a down beside the foam chin rest, cue "rest" and C/T for 1"-23" of chin contact. Gradually add visual and acoustic distractions. Build duration to 73 s, with and without distraction. Increments can be 1"–23", 26"–31", 34"–45", 50"–60", and 73"+.

2.1.8.1. Repeat this session until the dog will chin target for at least 73 s; each session should last between 5 and 15 min.

2.2. **Phase 2 – Mock MRI**.

NOTE: To assemble the stationary Mock MRI, compile a tunnel (proxy MRI bore, 70 cm diameter cardboard concrete form tube cut to a length of six feet and elevated on a three-foot high folding table), a plywood platform, an acrylic mock radio frequency (RF) receiver coil, the foam chin rest apparatus, and a speaker system (see **Table of Materials**). Stabilize the mock bore on the table with two plywood braces. Affix buffer pads affixed to the interior of the mock RF coil to provide padding against the dogs' ears and additional cranial stabilization/placement feedback.

2.2.1. *Introduce bore and elevation with reduced duration*. Conduct the entire first session with the mock bore on the ground. Cue the dog to enter the tunnel and lie down on the platform, C/T. Cue the dog to "rest" and C/T for any duration of chin targeting to the foam chin rest inside the bore on the ground.

2.2.1.1. Conduct subsequent sessions with the mock bore elevated 3'. Invite the dog to jump or lift the dog into the elevated bore, C/T.

2.2.1.2. Cue the dog to lie down, C/T.

2.2.1.3. Cue the dog to "rest" and C/T for 1–12 s of chin contact to the foam chin rest in the elevated bore.

2.2.1.4. Repeat this session until the dog will chin target for at least 16 s; each session should last between 5 and 15.

2.2.2. *Elevated chin rest with increasing duration.* Invite the dog to jump or lift the dog into the mock bore. Cue the dog to lie down, C/T.

2.2.2.1. Cue the dog to "rest" and C/T for 1–12 s of chin targeting to the foam chin rest in the elevated bore.

2.2.2.2. Build duration to 60 ss. Increments can be 1"–12", 16"–23", 26"–45", 60"+.

2.2.2.3. Repeat this session until the dog will chin target for at least 60 s; each session should last between 5 and 15 min.

2.2.3. *Introduce mock radiofrequency (RF) coil with no elevation and reduced duration*. While seated on the ground beside the mock RF coil and foam chin rest, C/T the dog for any investigation (i.e., sniffing, close proximity, orientation towards) of the apparatus.

2.2.3.1. Cue the dog to "rest" and use a nose touch or food lure to guide the dog's head into and through the mock RF coil onto the foam chin rest and C/T for 1–5 s of chin contact.

2.2.3.2. Build duration to 30 s. Increments can be chin rest for 1"–5", 6"–12", 16"–26", 30"+.

2.2.3.3. Repeat this session until the dog will chin target through the mock RF coil for 30 s; each session should last between 5 and 15 min.

2.2.4. Elevated chin rest in mock RF coil. With the mock RF coil and foam chin rest inside the mock bore, invite the dog to jump or lift the dog into the bore.

2.2.4.1. Cue the dog to lie down and “rest”, and C/T for 1–5 s of chin contact.

2.2.4.2. Build duration to 50 s. Increments can be 1”–5”, 6”–12”, 16”–26”, 28”–37”, 50”+.

2.2.4.3. Repeat this session until the dog will chin target to the foam chin rest through the mock RF coil in the elevated bore for 50 s; each session should last between 5 and 15 min.

2.2.5. Elevated chin rest in mock RF coil with increasing distraction and duration. With the mock RF coil and foam chin rest inside the mock bore, invite the dog to jump or lift the dog into the bore.

2.2.5.1. Cue the dog to lie down and “rest”, and C/T for 1–12 s of chin contact.

2.2.5.2. Gradually add visual and acoustic distractions. Build duration to 100 s (1’40”) with and without distraction.

2.2.5.3. Increments can be 1”–12”, 16”–37”, 41”–60”, 66”–88” (1’6”–1’28”), 100”+ (1’40”±).

2.2.5.4. Repeat this session until the dog will chin target for 100 s; each session should last between 5 and 15 min.

2.2.6. *Introduce ear padding, duration initially reduced.* Lift or invite the dog to jump into the bore, cue “down,” outfit him with ear padding, and C/T for any duration of tolerating the ear padding without excessive movement.

2.2.6.1. Cue “rest,” and C/T for 1–5 s of chin contact. Build duration to 60 s. Increments can be 1”–5”, 6”–26”, 28” 45”, 60”+.

2.2.6.2. Repeat this session until the dog will chin target in the mock bore with ear padding for 60 s; each session should last between 5 and 15 min.

2.2.7. *Elevated chin rest in mock RF coil with ear padding and increasing duration and distraction.* Lift or invite the dog to jump into the bore, cue “down” and “rest,” outfit him with ear padding, and C/T for 1–12 s of chin contact.

2.2.7.1. Gradually add visual and acoustic distractions. Build duration to 107 s. Increments can be 1”–12”, 16”–37”, 41”–60”, 66”–88”, 107”+.

2.2.7.2. Repeat this session until the dog will chin target in the mock bore with ear padding for 107 s; each session should last between 5 and 15 min.

2.2.8. *Introduce scanner noise.* Lift or invite the dog to jump into the bore, cue “down” and “rest,” and outfit it with ear padding.

2.2.8.1. Play scan audio at a barely audible volume between 0–40 dB and C/T for 1–12 s of chin contact.

2.2.8.2. Build duration to 107 s. Increments can be 1”–12”, 16”–37”, 41”–60”, 66”–88”, 107”+.

2.2.8.3. Repeat this session until the dog will chin target in the mock bore with ear padding and up to 40 dB scan audio for 107 s; each session should last between 15 and 30 min.

2.2.9. *Build duration to 2 min 30 s with increasing distance.* Lift or invite the dog to jump into the bore, cue “down” and “rest,” and outfit him with ear padding.

2.2.9.1. Play scan audio between 41 and 70 dB and C/T for 1–37 s of chin contact. Gradually add distance, moving around the mock bore, out of sight then back into sight of the dog.

2.2.9.2. Build duration to 142 s. Increments can be 1”–37”, 41”–88”, 97”–107”, 117”–129”, 142”+.

2.2.9.3. Repeat this session until the dog will chin target in the mock bore with ear padding and 41–70 dB scan audio for 142 s, with and without distraction and distance; each session should last between 15 and 30 min.

2.2.10. *Build duration to 4 min.* Lift or invite the dog to jump into the bore, cue “down” and “rest,” and outfit him with ear padding.

2.2.10.1. Play scan audio between 60 and 90 dB and C/T for 1–107 s of chin contact.

2.2.10.2. Build duration to 240 s. Increments can be 1”–107”, 117”–142”, 156”–189”, 208”–229”, 240”+.

2.2.10.3. Repeat this session until the dog will chin target in the mock bore with ear padding and 60–90 dB scan audio for 240 s, with and without distraction and distance; each session should last between 15 and 30 min.

2.2.11. *Build duration to 5 min*. Lift or invite the dog to jump into the bore, cue “down” and “rest,” and outfit it with ear padding.

2.2.11.1. Play scan audio between 80 and 110 dB and C/T for 1–120 s of chin contact.

2.2.11.2. Build duration to 300 s. Increments can be 1”–120”, 129”–189”, 208”–229”, 252”–277”, 300”+.

2.2.11.3. Repeat this session until the dog will chin target in the mock bore with ear padding and 80–110 dB scan audio for 300 s, with and without distraction and distance; each session should last between 15 and 30 min.

3. Transfer

NOTE: 1) Upon reaching final criterion of the stationing behavior in the mock MRI training location (5 min down-stay and chin rest in mock bore and mock RF coil while wearing ear padding, with scanner noise playing at 80-110 dB), the dog undergoes five distinct location transfer (generalization) sessions. During these transfer sessions the dog stations to the above criteria in several indoor and outdoor locations that are as unique as possible, with different sights, sounds, and degrees of social distraction across settings (e.g., secluded grass field, quiet academic building hallway, busy academic building lobby, crowded bus stop, loud water treatment plant)[8].

3.1. To assemble the portable Mock MRI used for Transfer, compile a tunnel (regulation agility tunnel, stabilized with sand bags), a plywood platform, an acrylic mock RF receiver coil, the foam chin rest apparatus, and a portable speaker system (see **Table of Materials**). Affix buffer pads affixed to the interior of the mock RF coil to provide padding against the dogs' ears and additional cranial stabilization/placement feedback.

3.2. Stand or sit beside the (portable) mock bore with the mock RF coil and chin rest inside. Gesture the dog to enter the bore, cue "down" and "rest," and outfit it with ear padding.

3.3. Play scan audio at 80–110 dB and C/T for 1–30 s of stationing in the new location.

3.4. Next, probe for criterion duration (reinforce at 5 min or when the dog breaks).

3.5. Conduct repeated bouts of 1–5 min chin rest repetitions for 30–60 min.

3.6. Conduct one 30–60 min session in each location. Once the dog has generalized the stationing behavior to criterion in five distinct transfer locations, the dog is ready for data collection in the real MRI environment.

4. MRI

4.1. An MRI data collection session takes between 30 and 60-min. Begin the data collection session with a localizer, followed by acquisition of high-resolution anatomical images to serve as a prescription reference using the 3D magnetization-prepared rapid gradient echo (MPRAGE) sequence. Following the anatomical scan, perform the functional Magnetic Resonance Imaging scans (e.g., stimulus presentation and resting state). This MRI protocol represents examples from prior work and is only suggestive (see Jia et al.[4] and Thompkins et al.[10] for how to perform the protocol).

4.1.1. Give the dogs approximately 1 min breaks with their reward outside of the scanner between scans.

REPRESENTATIVE RESULTS:

The mean number of repetitions of each session level is listed in **Table 1**. The complete training and testing protocol required 14 h (M = 13.55 h, range 12–16 h) and consisted of 90 sessions (range 87–93 sessions). Open environment training lasted 4.38 h (range 3–5 h), mock MRI training lasted 5.4 h (range 4.2–6.5 h), and transfer was 2.5 h divided into five 30 min sessions. Maintenance sessions at level 19 were conducted during transfer and are reflected in the complete training time above.

Stationing training and testing

Figure 3 shows the maximum duration of four dogs trained in the protocol for the last three sessions at the end of training and the different training locations. Performance was stable at the end of stationing training, $F(2, 6) < 1$, and over 5 min ($M = 311$ seconds, SEM = 1.9). All dogs transferred to the mock training locations with a max duration equivalent to training, $F(1,3) < 1$. Three of the dogs transferred to the MRI scanner and demonstrated repeated bouts of the max possible duration (206 s). The one dog that did not transfer to the MRI scanner had a larger head than the other dogs and could not comfortably fit within the coil. This discomfort likely led to the dog not willingly participating in the scans.

[Place **Figure 3** here]

Representative fMRI stimulus driven scans

In these scans, visual or odor stimuli were presented to the dog while the dog remains still. Visual stimuli were projected on a screen located in the bore of the scanner. Each scan lasted for 140 s and contained 12 different images (e.g., human and dog faces). A stimulus was presented for 5 s followed by a variable 3–11 s inter-stimulus interval (see **Figure 4** for visual depiction and Thompkins et al. 2018 for additional details)[10].

[Place **Figure 4** here]

Attentional check

To determine whether dogs were attending to the visual stimuli, independent raters viewed videos of the dogs' eyes inside the bore of the MRI scanner synced up with the stimulus presentation. Based on whether the dogs' eyes were open and their pupils visible, the raters assigned an appropriate score for each stimulus (**Figure 5**). fMRI data was used only when there was perfect inter-rater agreement.

[Place **Figure 5** here]

Dog and human face processing

Figure 6 shows adjacent but different brain areas of temporal cortex in the dog brain are active for processing dog and human faces. Green regions represent areas of the brain more active for human faces contrasted with dog faces ($p < 0.05$, FDR (false discovery rate corrected)). Red regions represent areas of the brain more active for dog faces contrasted with human faces ($p < 0.05$, FDR).

[Place **Figure 6** here]

Odor stimuli

Odor stimuli were delivered through an olfactometer (**Figure 7**); high (0.16 mM) and low (0.016 mM) concentrations of odorant ethyl butyrate were used to probe parametric modulation of olfactory areas by odorant concentration. Each scan lasted 200 s and contained 5 blocks of 10 s odorant stimulation, each followed by a 30 s inter-stimulus interval (see **Figure 8** for visual depiction and Jia et al. 2014 for additional details)[4].

[Place **Figure 7** here]

[Place **Figure 8** here]

Researchers delivered high (0.16 mM) and low (0.016mM) concentrations of an ethyl butyrate solution to six trained detection canines (Labradors) while awake and anesthetized. The parametric increases in magnitude of activation to low and high concentrations of odorant in olfactory regions (olfactory bulb, bilateral piriform lobes, cerebellum) was in accordance with Weber's Law (threefold perceived increase for a tenfold concentration increase). In addition, while the olfactory bulb, periamygdala, anterior olfactory cortex, entorhinal cortex, and piriform lobes were active in both awake and anesthetized dogs, regions implicating higher-order cognitive processing (superior, medial and orbital portions of frontal cortex) were activated mainly in awake dogs (**Figure 9**).

[Place **Figure 9** here]

FIGURE AND TABLE LEGENDS:

Figure 1. Protocol timeline. The protocol timeline is divided into two components, Training and Testing. Training is further divided into two phases, Open Environment and Mock MRI. Separate Auditory Exposure sessions occur during training as well. Testing consists of stationing in a portable mock MRI, in five different transfer locations (T1–T5). Once the dog has generalized the stationing behavior to criterion in five distinct transfer locations, the dog is ready for data collection in the real MRI environment.

Figure 2. Active Exposure. Active Exposure (AE) is a short-delay classical conditioning procedure. 10 s CS (i.e., scan audio presented by itself), 20 s CS + US (i.e., ball and scan audio presented together), 10 s delay (no ball, no scan audio). After this delay, the trial starts over. There are ten trials per session, with incremental volume increases over sessions.

Figure 3. Maximum duration of four dogs trained in the protocol for the last three sessions at the end of training and the different training locations. All dogs transferred to the mock training locations and three of the dogs transferred to the MRI scanner demonstrating the maximum possible duration (206 s).

Figure 4. Visual stimuli. The top panel shows an example run of dog faces. The bottom panel shows an example run of human faces. Face stimuli were displayed for 5 s, with 3–11 s inter-stimulus intervals. Twelve face stimuli were shown per run.

Figure 5. Attentional check. To ensure each dog looked at each stimulus presented during scanning, stimulus-synchronized video of the dog's eye inside the scanner was analyzed by two raters post hoc; for each trial, if the dog's eye was visibly open, the rater assigned a score of "yes" and if the dog's eye was closed, the rater assigned a score of "no." fMRI data was used only when there was perfect inter-rater agreement. This figure has been modified from Thompkins et al.[10]

Figure 6. **Results of human and dog face contrasts.** Regions in green represent areas that are significantly more active during processing of human faces as compared to dog faces (i.e., human face area, HFA). Regions in red represent areas that are significantly more active during processing of dog faces as compared to human faces (i.e., dog face area, DFA). This figure has been modified from Thompkins et al.[10]

Figure 7. Olfactory imaging system. Components of the dog olfactory imaging system outside the MRI room showing odorant applicator, air tank, motion parameter recording palmtop, video monitor, laptop with VT-8 software, and the entrance port to the MRI room. This figure has been modified from Jia et al.[4]

Figure 8. Odorant delivery. Odorant delivery was controlled by VT-8 Warner Timer software in a fMRI block design. The first row shows the odorant delivery sequence with green arrows indicating stimulus onset and red arrows indicating stimulus offset. The second row shows clearance of odorant, with green arrows indicating onset of odorant clearance and red arrows indicating offset of odorant clearance. The third row shows the fMRI block design, matching the first row, with "0" and "1" denoting odorant "off" and "on" conditions, respectively. This figure has been modified from Jia et al.[4]

Figure 9. Group activation maps for anesthetized dogs. Three orthogonal views are shown in each subfigure. Colormap is used for activation intensity and important areas are indicated by arrows with labels (Overall FDR = 0.05, cluster threshold = 15 voxels using AlphaSim, t-contrast). A: Anterior, P: posterior, S: superior, I: inferior, L: left, R: right. Subfigure (**A**) corresponds to low concentration odorant (0.016 mM), subfigure (**B**) corresponds to high concentration odorant (0.16 mM), subfigure (**C**) corresponds to anesthetized dog olfactory processing, and subfigure (**D**) corresponds to awake dog olfactory processing. This figure has been modified from Jia et al.[4]

Table 1. Session levels. *See note in manuscript. **Conduct first session with mock bore on ground.

DISCUSSION:

The protocol described above separates the training of the stationing (chin rest) behavior from desensitization to the MRI environment. Further, it utilizes a generalization procedure of stationing in several dissimilar locations, to assist in the transfer of the stationing behavior to the real MRI scan environment; it does so without the need for extensive training time in the MRI scan environment, which can be expensive. Overall, the training and testing was completed in 14 hours and resulted in immediate transfer to novel locations. Although it is difficult to compare methods across laboratories in a meaningful way at this time, we present a canine fMRI training protocol that can be completed in a cost-effective manner, with high-energy dogs.

Regarding the generalization of this training protocol to other trainers, while we used kenneled purpose-bred detection dogs, this protocol should bode well for other dog populations. Detection dogs are typically repurposed American Field Trial, Hunt Test, and Upland Game dogs with intrinsically high energy and "high drive"[11]. The term "drive," referring to the dog's intrinsic motivation to work, is both difficult to operationalize and measure, and widespread in its use to characterize dogs that are most suitable for detection work; the industry favors and selects for bold, excitable, high-energy dogs, with higher baseline levels of arousal (i.e., excitement, anxiety) than other types of working dogs and pets[12]. If such dogs can be trained to station, other populations should be successful too. Further, dogs were able to station in a variety of locations, including the real MRI scanner. As for pet dogs, whether or not the training protocol can be successful is an empirical question. Notably, with proper instruction, all of the training steps can be implemented in the home environment with the portable mock bore.

Clicker training, successive approximation, and classical-counter conditioning are methods used to condition behavior in a diverse range of species, from laboratory mice to wild animals in captivity[8]. The methods are forgiving with respect to small mistakes made throughout the training process (e.g., marking and reinforcing the wrong behavior, lack of interest in the reward)[13]. The same dimensions that make the methods more forgiving for novice teachers also make them more universal to the animal learners; by increasing the success rate of more dogs in the subject pool and more types of dogs (e.g., special population detection dogs), one can begin to combat an inherent selection bias due to subject and potentially reduce data attrition. This bias afflicts experimental samples and stems from an inability of the method to adapt to individual variability in temperament and tractability for an apparatus-oriented task that necessitates high levels of patience and impulse control, as is required for stationing for MRI. In reducing attrition, this method provides support for two out of the three R's (replacement, reduction, and refinement) for the best practices of experimental design with animal subjects[14]. Fewer subjects are needed when fewer of the procured subject pool are expected to attrite out of training, and fewer scan sessions are needed with less of the data having to be censored out due to high amplitude or frequent motion artifacting, a potentially noteworthy *reduction*. The training minimizes pain and distress of the animal learner in acquiring this task, a potentially noteworthy *refinement*.

The training materials are easily and inexpensively procured. The classical counter conditioning and generalization elements of this method reduce stress and novelty of the scanner environment, without the need for several expensive training hours in a rented scanner environment. Without training in the MRI environment, the trainer is unable to replicate the static magnetic field within the scanner bore or the unusually high/low frequency audio emissions of the scan sequences; this limitation is potentially addressed because theoretically, these dimensions are lumped into a 'variability' component from performing the behavior in diverse settings during training.

Another limitation is that this protocol is not optimized for speed. The stationing behavior can be conditioned in thirteen hours of training, which over six months equates to approximately 80 five to fifteen-minute training sessions. The methodological approach is "slow and correct," instead of "fast and fix it later". A mentality of "fast and fix it later" leads to potential sensitization of the scanning environment, and subsequent attrition of data or entire subjects. In a study of lexical processing in dogs, researchers were able to collect data at a success rate of 80% on a given dog's first attempt. If a dog needed a second attempt, success dropped to 16%, and just 4% if the dog needed a third attempt, suggesting that those dogs became detrimentally sensitized to the scanning environment with repeated exposure[15]. The protocol described above will likely not work on all dogs, and methodological alternatives include using a nose-to-target stick behavior instead of a chin rest behavior, implementing longer training sessions, and increasing the frequency of the training sessions. One could pre-screen for more suitable subjects (e.g., tractability, temperament, head size), although the trade-off with selection bias persists, and further, the more difficult-to-train dogs might be model pathologies of interest: disordered anxiety, aggression, special population working dogs (e.g., those selected for high-drive/energy). To better compare methods of acclimating and training for stationing in the MRI, we would need more dogs and more trainers. A strength of the work done at Auburn University is the researcher's access to the "Auburn Dog" population through Canine Performance Sciences (CPS). The detection dogs used in these studies have similar genetics and near-identical rearing and training histories.

Independent of training method, certain technological improvements could enhance the fidelity of canine MRI data, including improved radiofrequency coil design to facilitate imaging of canine cranial anatomy, as well as improved hardware and sequences to quiet the scanner during data acquisition[7]. Awake, unrestrained fMRI has provided cognitive insights into the canine psyche with respect to multimodal learning, executive function, stimulus processing, and reward processing[6,16-27]. Comparative and translational researchers can examine multiple sensory modalities with this imaging technique. The technique can be used to probe information processing in special working populations (e.g., signal processing in service dogs and parametric odor processing in detection dogs)[4,28]. These techniques have translational utility when it comes to determining operational potential and suitability to a working role; as a convergent technique alongside genetic and behavioral analyses, information gained from MR stimulus-presentation paradigms can inform selection of suitable working dog phenotypes for breeding purposes.

Many training strategies come from marine mammal and zoo animal training practices, adapted from Skinner, to approximate husbandry procedures via reinforcement-based enrichment and training[8]. Routine veterinary procedures (weight-taking, nail clipping, blood draws), or anything uncomfortable or anxiety-provoking, can be facilitated with reinforcement by successive approximation following a dedicated training plan, modeled after the one suggested here for canine fMRI. Awake, unrestrained MRI has even been discussed as having clinical utility in its own right for epileptic dogs[29].

In summary, canine fMRI is in its nascent stages. We have presented a humane training program that can be successfully implemented in a cost-effective manner. The future is promising for the continued use of “man’s best friend” in understanding, brain-behavior relationships as the field of cognitive neuroscience continues to evolve.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure 1

<table>
<tr><th colspan="2">Training</th><th colspan="6">Testing</th></tr>
<tr><td colspan="2">Auditory Exposure (PE then AE)
(~20 weeks)</td><td colspan="5">Transfer
(5 weeks)</td><td rowspan="2">MRI</td></tr>
<tr><td>Phase 1
Stationing in Open Environment
(~ 10 weeks)</td><td>Phase 2
Stationing in
Mock MRI
(~10 weeks)</td><td>T1</td><td>T2</td><td>T3</td><td>T4</td><td>T5</td></tr>
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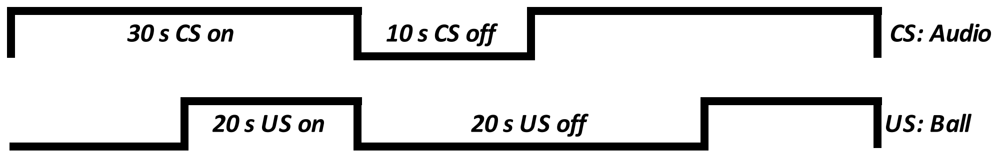
Figure 2



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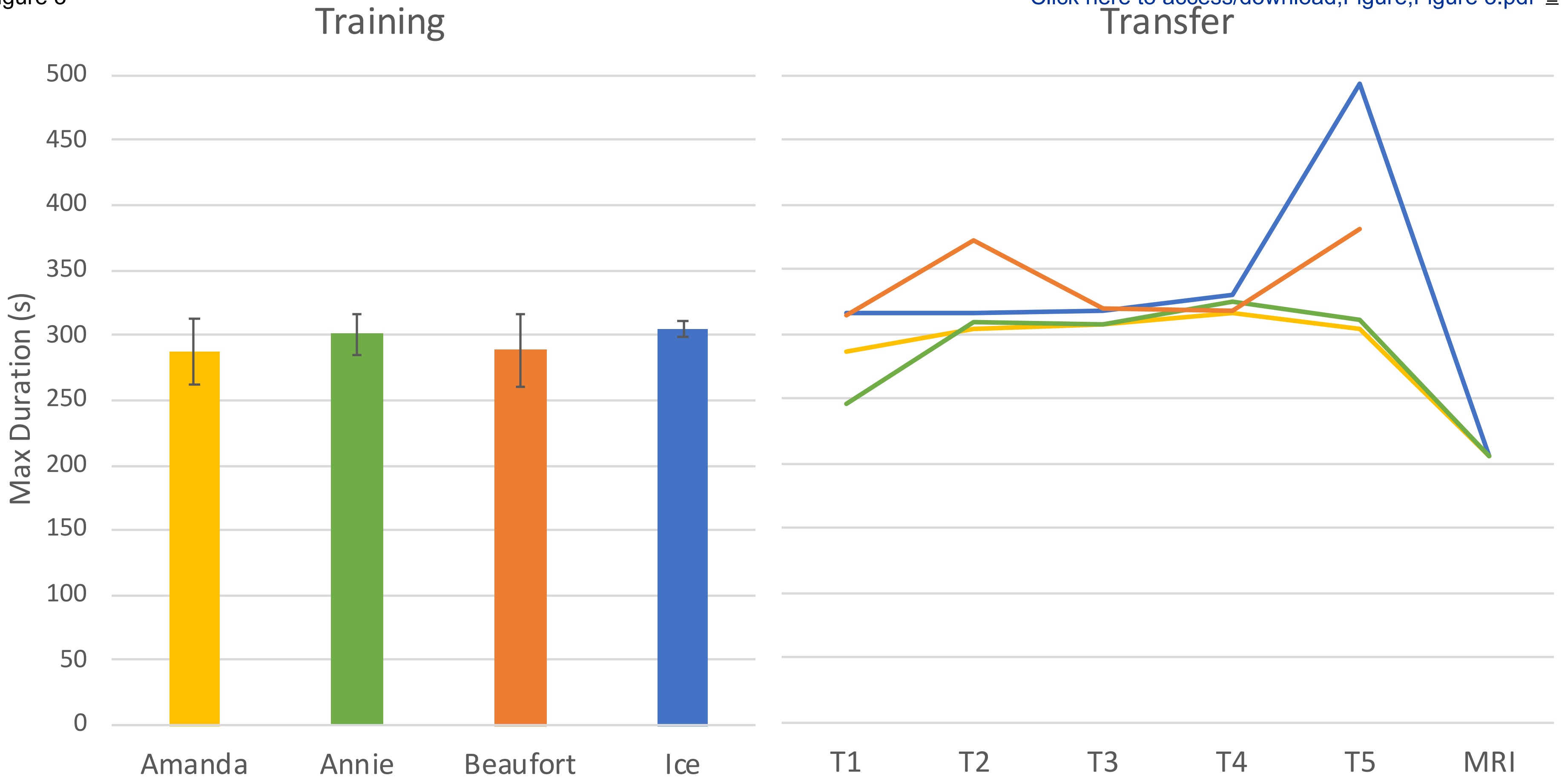
Training
Transfer
Max Duration (s)
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300
250
200
150
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Annie
Beaufort
Ice
T1
T2
T3
T4
T5
MRI

Figure 4

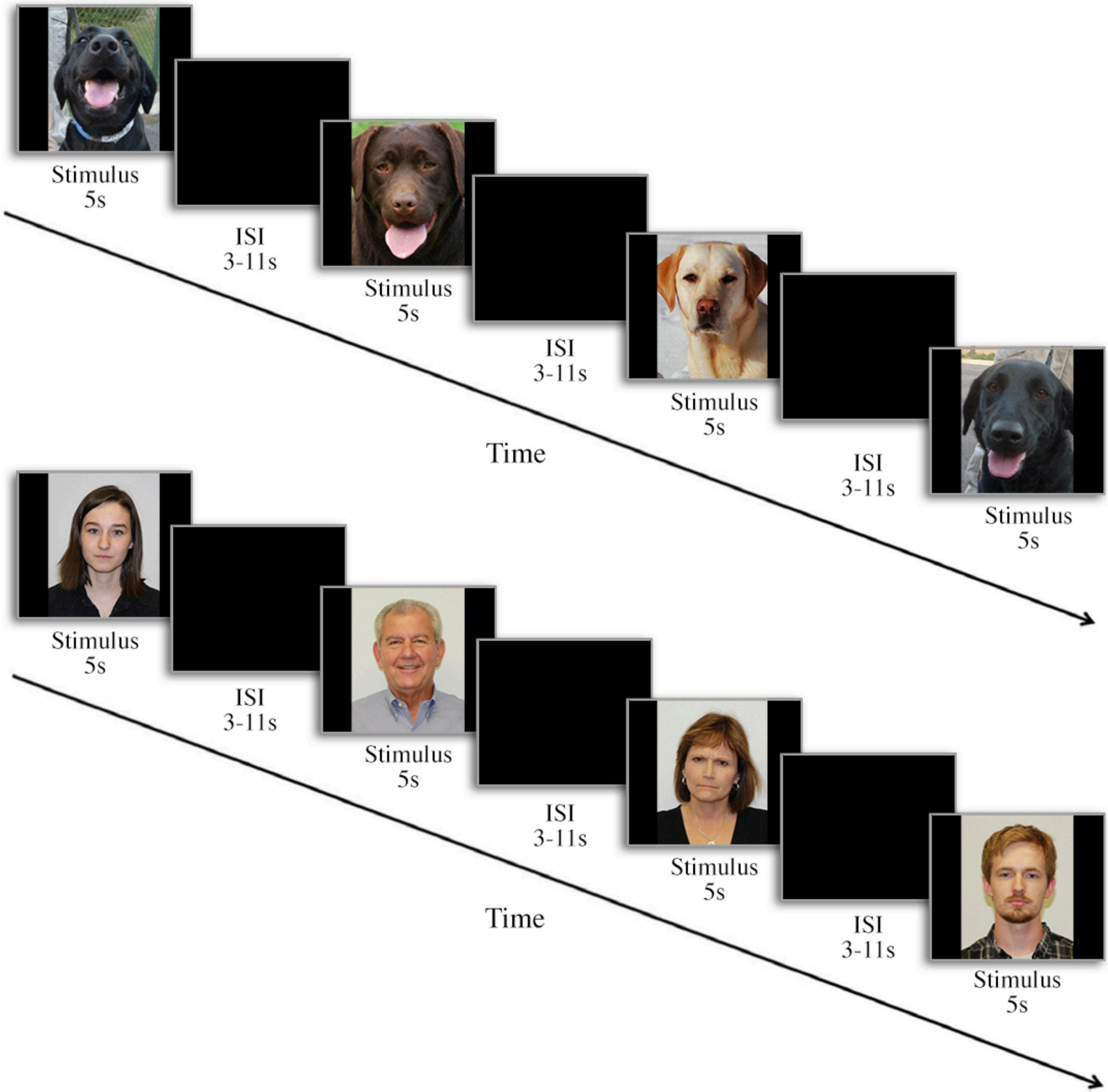
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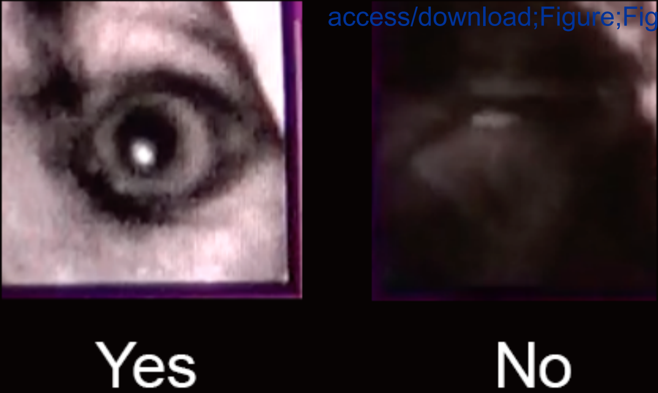
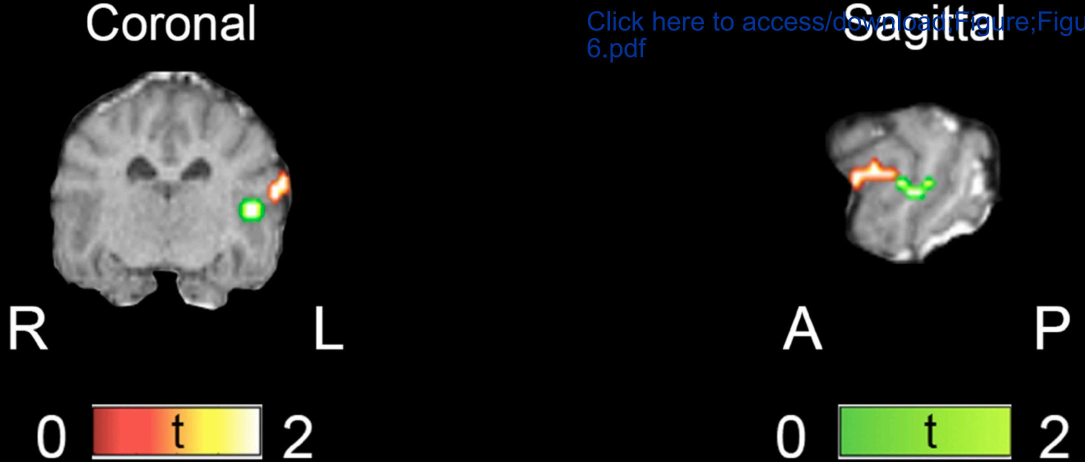
Figure 5

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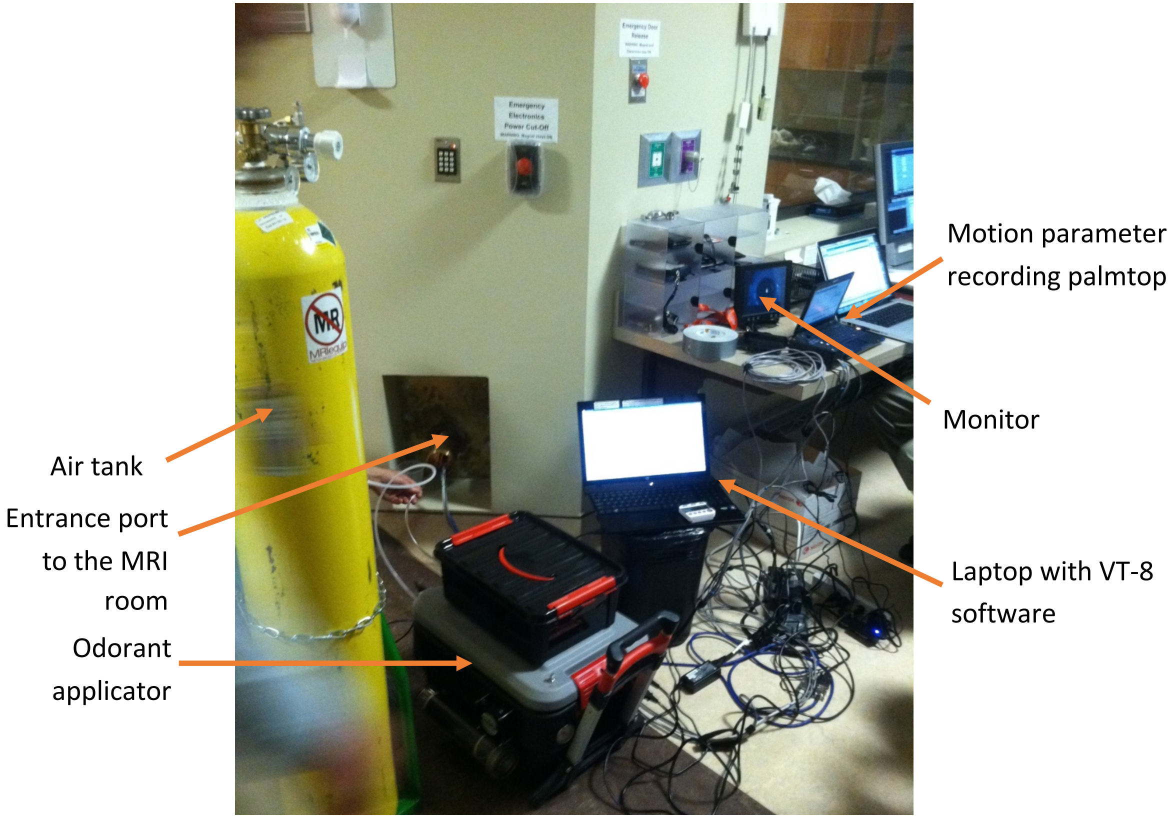

Motion parameter recording palmtop
Monitor
Air tank
Entrance port to the MRI room
Laptop with VT-8 software
Odorant applicator

Figure 8

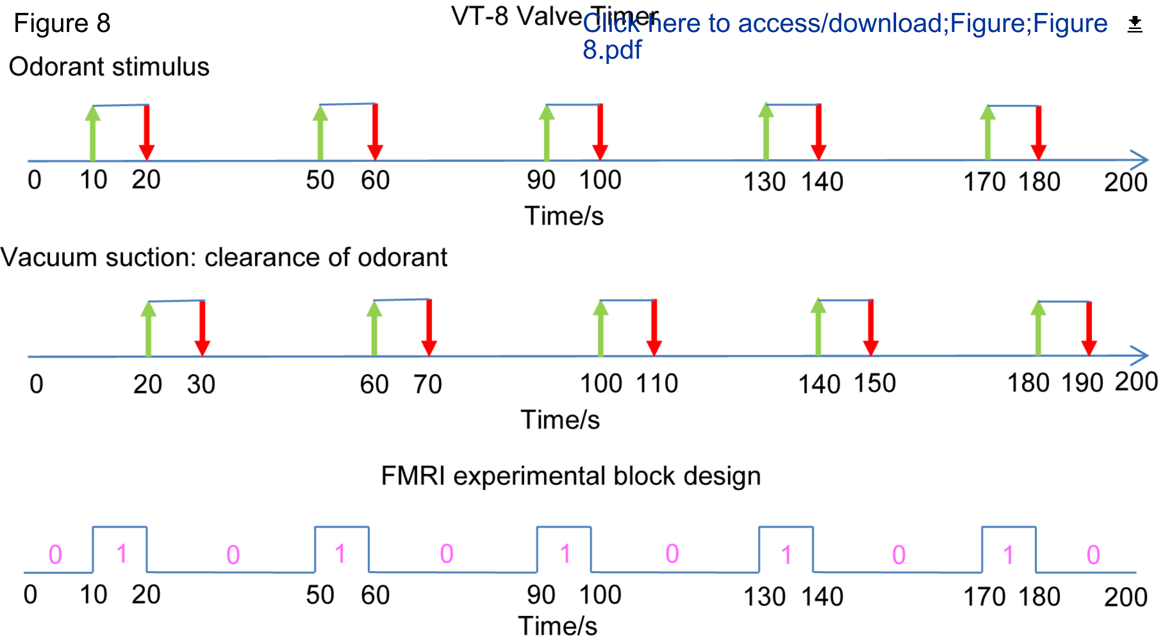
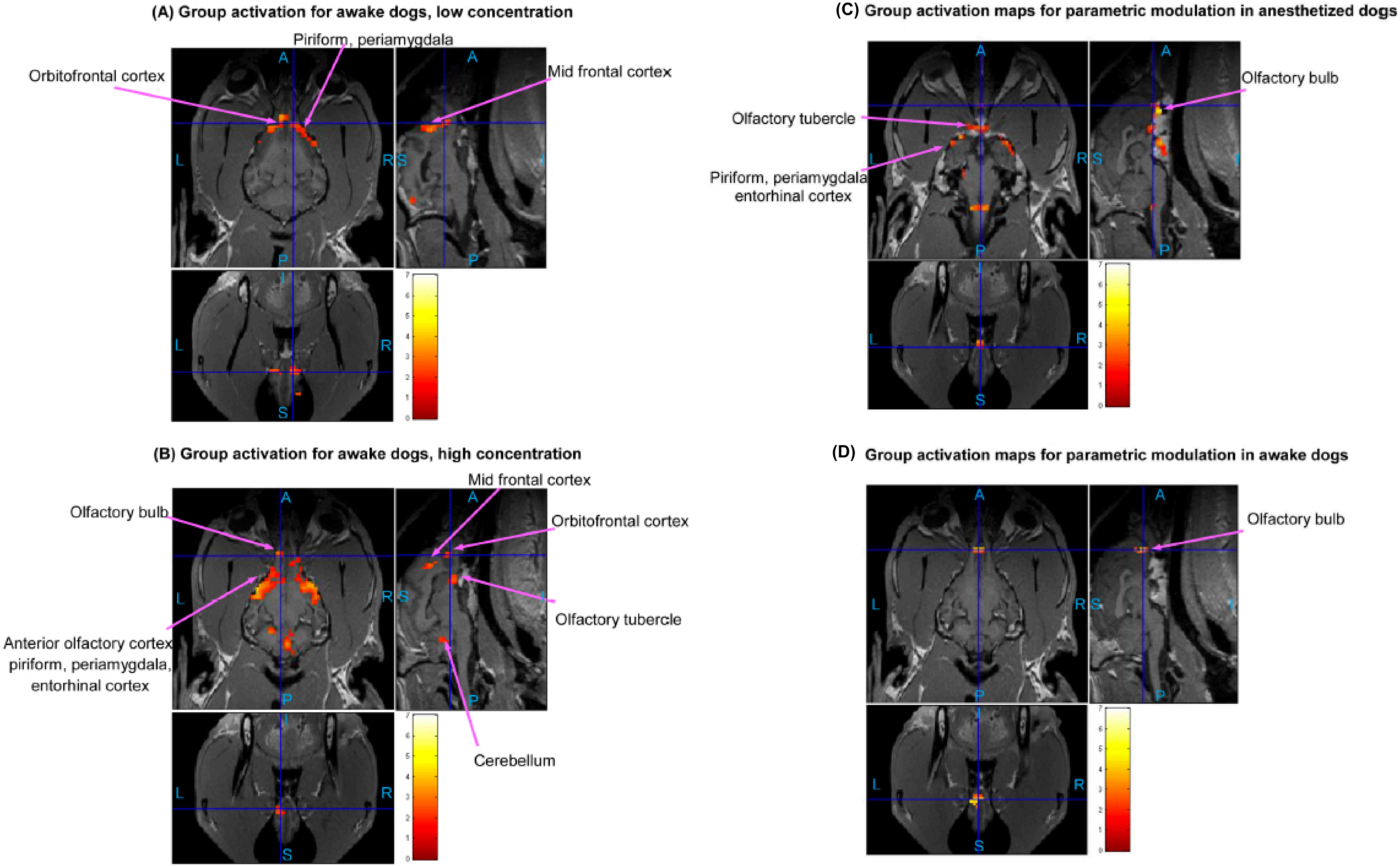

VT-8 Valve Timer
Odorant stimulus
0 10 20 50 60 90 100 130 140 170 180 200
Time/s
Vacuum suction: clearance of odorant
0 20 30 60 70 100 110 140 150 180 190 200
Time/s
FMRI experimental block design
0 1 0 1 0 1 0 1 0 1 0
0 10 20 50 60 90 100 130 140 170 180 200
Time/s

Figure 9

Click here to access/download;Figure;Figure 9.pdf



	Session Level
Open Environment	1. Charge the clicker
	2. Capture chin target to towel
	3. Chin-to-towel target with short duration and adc
	4. Chin rest on towel in a down and addition of dist
	5. Chin rest on towel with distance
	6. Chin rest on towel with increasing duration and (
	7. Introduce foam chin rest, duration initially reduc
	8. Chin rest in foam chin rest with increasing durati
Mock MRI	9. Introduce bore and elevation with reduced dura
	10. Elevated chin rest with increasing duration
	11. Introduce mock radiofrequency (RF) coil with n
	12. Elevated chin rest in mock RF coil
	13. Elevated chin rest in mock RF coil with increasir
	14. Introduce ear padding, duration initially reduce
	15. Elevated chin rest in mock RF coil with ear padc
	16. Introduce scanner noise
	17. Build duration to 2 minutes 30 seconds with inc
	18. Build duration to 4 minutes
	19. Build duration to 5 minutes
Transfer	20. Five distinct location transfer (generalization) s
All	Final behavior(s)

Criteria

Build an association between the 'tic-toc' of the clicker and the dog's primary reward (e.g., food) while capturinga

Build chin-to-towel contact to 2+ seconds. *

Chin contact for 7+ seconds. *

Chin contact for 11+ seconds, with and without distraction.

Chin contact for 16+ seconds, cued from progressively farther away (i.e., sitting on ground, kneeling, standing).

Chin contact for 26+ seconds.

Chin contact to foam chin rest for 40+ seconds.

Chin contact for 73+ seconds.

Chin contact in bore on table for 16+ seconds. **

Chin contact in bore on table for 60+ seconds.

Chin contact in RF coil on ground for 30+ seconds.

Chin contact to foam chin rest through the mock RF coil in the elevated bore for 50+ seconds.

Chin contact for 100+ seconds, with and without distraction.

Chin contact in mock bore and RF coil (mock MRI) with ear padding for 60+ seconds.

Chin contact for 107 seconds, with and without distractions.

Chin contact in mock MRI with ear padding and up to 40 dB scan audio for 107+ seconds.

Chin contact in mock MRI with ear padding and 41-70 dB scan audio for 142+ seconds, with and without distractio

Chin contact in mock MRI with ear padding and 60-90 dB scan audio for 240+ seconds, with and without distractio

Chin contact in mock MRI with ear padding and 80-110 dB scan audio for 300+ seconds, with and without distract

During these transfer sessions the dog stations to the above criteria in several indoor and outdoor locations that a

The dog performs a chin rest with head and body enclosed at a 3' elevation, with ear padding and scan audio play

Duration	Session Repetitions (M, SE)
3 min	1, 0
5 min	3.75, .75
5 min	8.25, 2.8
5-10 min	2.75, .25
5-10 min	3.5, .87
5-10 min	5.5, 1.5
5-15 min	4.75, .75
5-15 min	6, 1.2
5-15 min	2.5, .5
5-15 min	3, 0
5-15 min	2.75, .25
5-15 min	2, 0
5-15 min	2.5, .29
5-15 min	3, .41
5-15 min	2.5, .29
10-30 min	2.5, .5
10-30 min	2.5, .5
10-30 min	2.75, .75
10-30 min	10, 1.8
30 min	5, 0
12-16 h (M	87-93 sessions (M=90, SE=1.5)

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Acrylic Mock Radiofrequency Coil	Menards	TU59018594	Mock Radiofrequency (RF) Coil: 8" diameter x 4' Concrete Form Tube. Makes four mock RF coils; cut form tube in four even lengths for four 8" diameter x 1' mock RF coils.
Agility Tunnel	J&J Dog Supplies	TT053	Open Agility Training Tunnel
Bluetooth Speaker	Sharkk	SP-SK896WTR-GRY	Portable Scan Audio Playback: Waterproof Bluetooth Speaker Sharkk 2O IP67 Bluetooth Speaker Outdoor Pool Beach and Shower Portable Wireless Speaker
Cardboard Concrete Form Tube	Menards	TU10120014	Stationary Mock MRI Bore: Sonotube 24" diameter x 12' Standard Wall Water-Resistant Concrete Form. Makes two mock bores; cut form tube in half for two 24" diameter x 6' bores.
Chuckit Ball	Chuckit!	17030	Toy Reward: Chuckit! Ultra Ball
Decibel X	Skypaw		Decibel meter phone app
Exercise Mat			Foam chin rest: cut mat in half lengthwise. Roll up, and secure roll with hot glue. Cut chin-size notch in center with X-ACTO knife. Hot-glue velcro to bottom surface.
Folding Table			3' x 6' folding table
Microfiber Car Wax Applicator Pad	Viking Car Care	862400	Viking Car Care Microfiber Applicator Pads
Natural Balance Treat Log	Natual Balance	236020	Food Reward: E.g., Chicken Formula Dog Food Roll, 3.5-lb roll
Plywood			Platform: 2"x4"x6' length of wood affixed to 3'x6' plywood board. Hot glue exercise mat on plywood board for traction. Braces: 3 4x4x4" cubes cut at 45-degree angle affixed to ends of 1"x4"x3' lengths of wood. Makes 3 braces.
Sand Bags	J&J Dog Supplies	AG155	J&J Professional Quality Sandbags x 2

Speaker System	Pioneer Electrics	HTD645DV	Stationary Scan Audio Playback: Pioneer HTD645DV 5 Disk DVD Home Theater System with Wireless Surround Speakers. Operating Instructions: https://www.pioneerelectronics.com/pio/pe/images/portal/cit_11221/93327312Operating%20Instructions%20S-HTD630.pdf
Towel			standard towel

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Below are the reviewer comments and specific responses regarding manuscript JoVE60192 entitled *Training Dogs for Awake, Unrestrained Functional Magnetic Resonance Imaging* for JOVE: Journal of Visualized Experiments. Our responses are in **bold** and follow the order of the action letter. We appreciate the time and effort of the reviewers and their many excellent suggestions which have all improved the manuscript.

Editorial Comments:

"Please split up longer Protocol steps so that individual steps contain only 2–3 actions (in the imperative) and a maximum of 4 sentences."

We have edited accordingly.

"Please only highlight protocol steps for filming; the results will be handled separately."

We have edited accordingly.

"For each protocol step, please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. If revisions cause a step to have more than 2-3 actions and 4 sentences per step, please split into separate steps or substeps."

We have edited accordingly.

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This has been done.

"Table of Materials: Please ensure the Table of Materials has information on all materials and equipment used, especially those mentioned in the Protocol."

We have reconciled the Table of Materials with all materials mentioned in the manuscript.

Reviewer 1:

"Magnetic resonance imaging (MRI) conducted on unrestrained awake dogs is a new method" - How new? Please, accompany with citations."

We have included the information on lines 61-62.

"There are several advantages of studying brains in awake dogs for functional magnetic resonance imaging (fMRI)." - Odd wording. Do you mean there are several advantages OF functional magnetic resonance imaging for studying brain function in awake dogs?"

We have clarified this statement on lines 62-63.

"enhancing comparative neuroimaging" - in what way? This is a bit nondesript."

On lines 63-64 we have edited to reflect that cross-species comparisons are more generalizable across dogs and humans because both species are tested awake and unrestrained.

"At the moment there are five laboratories around the world" - which are these?"

These are now mentioned on lines 71-72.

"The Authors talk about unrestrained scanning and awake scanning being advantageous but only present specific ideas on the latter. It may be helpful to mention advantages to the former as well, e.g., fluid or food deprived or physically restrained organisms are likely very different in their cognitive states than their non-deprived and unrestrained counterparts, see, for example, review by Bunford et al. 2017 TiNS."

Thank you for the suggestion, we have added the deprivation point and reference on lines 67-69.

"the purpose of being cost-effective, producing quality data, and generalizable to trainers across the world" - I recommend the Authors revise this sentence for parallel construction."

We have edited the sentence (Lines 79-81).

"This protocol can be completed in 25 weeks and can be shortened depending on the frequency of training sessions" - Can we be certain that different completion durations does not have any effect on learning or performance? I again appreciate that this is a very strict criterion but the whole purpose of the current protocol, as articulated by the Authors, is that it is standardized."

This is a good point and we have decided to not use the word standard, as it is clear there are too many factors to work out before standardization can occur. Although, our intention is to move and inspire the field in that direction. We have also removed the "shortened" phrase.

"On non-training days, the dog is usually transported to a familiar exercise area and allowed to play. General obedience is also practiced on these days (e.g., downs and stays in exchange for treats and toy tosses)." - this presumes the dogs that can be trained with this protocol or that the dogs that this protocol is applicable to are not family dogs but are lab dogs. If this is true, then this should be noted as a limitation to generalizability across research labs as some labs work with family dogs."

We have added a section (lines 677-689) in the discussion on generalizability of the training protocol. As for pet dogs, we believe the training procedures is generalizable to pet dogs but it is a limitation that will need to be determined by future studies.

"What is the size of the chin rest apparatus and how is it adjusted to any given dog's size?"

On lines 144-1146 we describe the size and adjustment of the chin rest apparatus: The chin rest was 6 in wide with a 4 in wide x 2.5 in deep notch for the muzzle. It was designed to fit each individual that we working with, all approximately the same size/breed.

"whatever the dog prefers can be used initially, even if it is high-motion play (e.g., ball, tug)." - the Authors may wish to make note of some dogs being more motivated by food whereas others being more motivated by play or praise, see, e.g., Gerencsér et al. 2018. Sci Rep."

Thank you for the suggestion, we have edited accordingly.

"and the dog's primary reward (i.e., food) while capturing attention" - I take it the i.e., should be replaced with e.g.,."

We have edited accordingly.

"I am not sure what the yellow highlighting indicates in this manuscript."

This is standard for JoVE to indicate aspects of the manuscript to be video recorded.

"A flow diagram of the different training and testing phases, with brief description of content, completion criteria, and number and duration of sessions would be very helpful and informative"

Thank you for this suggestion. We have added the requested information to the manuscript in Table 2.

"Each MRI data collection session takes between 30 to 60-minutes. All scanning takes place in the Siemens Verio open-bore 3T MRI scanner at the Auburn University MRI Research Center." - I am not sure why this site-specific information is presented in this manner, it makes it appear as if anyone who wishes to use this protocol has to scan at AU."

We have removed this information. Upon reflection we have removed specifics about the scanning sessions, as we are not proposing these particular MRI scans or parameters are followed. We have referenced the appropriate articles where readers can read about the information if they are interested.

"Same goes for scanning procedures - is it necessary to start with the structural measurement for this protocol to work? and is it necessary to use the MPRAGE sequence? I think the Authors should differentiate between what aspects of the protocol are generalizable vs. which ones are example they use to describe their own procedures at their site."

We agree. We have removed some details and state on lines 525: "This MRI protocol represents examples from prior work and is only suggestive (see references[4,10] for how to perform the protocol)."

"The Authors make note of their protocol being useable with high-energy dogs but it is unclear what they mean by this and what makes this protocol suited for use with high-energy dogs."

We have added the following information (Lines 679-684):

"Detection dogs are typically repurposed American Field Trial, Hunt Test, and Upland Game dogs with intrinsically high-energy, and "high-drive". The term "drive," referring to the dog's intrinsic motivation to work, is both difficult to operationalize and measure, and widespread in its use to characterize dogs that are most suitable for detection work; the industry favors and selects for bold, excitable, high-energy dogs, with higher baseline levels of arousal (i.e., excitement, anxiety) than other types of working dogs and pets."

We also included two references where we have discussed the topic for the interested reader.

> **Lazarowski, L. *et al.* Investigation of the Behavioral Characteristics of Dogs Purpose-Bred and Prepared to Perform Vapor Wake® Detection of Person-Borne Explosives. *Frontiers in Veterinary Science.* 5 (50), (2018).**
>
> **Lazarowski, L., Waggoner, P. & Katz, J. S. The future of detector dog research. *Comparative Cognition & Behavior Reviews.* 14 77-80, (2019).**

<u>Reviewer 2:</u>

"The only somewhat conceptual point I would want to raise is that the authors should somewhat tone down claims that their training protocol and its reliance on generalization is "better, more efficient/effective" than other ones used in the field. This is not because I do not think that this is a plausible claim to be made, but because there are no data presented (and they would in fact be hard to procure anyways, as the time and monetary costs for a study providing direct comparisons of training with and without generalization, while keeping everything else constant, seem hardly justifiable) to substantiate this claim."

We agree and have revised the manuscript accordingly.

"Clarification on ear protection: I did not fully understand what the ear protection entails. Only on ear protection, or also in ear protection. Maybe also mention how the protection used ensures protection against hearing damage."

We've used buffer pads that we believe provide some ear protection but have not been tested for this purpose, so we have removed the phrase ear protection from the manuscript and replaced with padding.

“Clarification on TE: The TE used seems somewhat lower to what seems a standard TE at 3T in human fMRI, though my experience is mainly with TIM Trio, Prisma, and Skyra. Please clarify why you use such a TE, and based on what considerations.”

We have removed this information and focused the protocol on the training, not the imaging protocols.

“Figures: Maybe I missed this but please clarify what images are used for figures showing brain anatomy; am I assuming correctly that these are overlay of SPMs (fMRI activation) on structural scans? From the same dog? From a standard template? If so, which one? Relatedly, consider showing EPIs across the whole brain so readers can evaluate the actual fMRI scanning resolution as well as the extent of signal drop out/lack of brain coverage in some areas.”

These are excellent and important suggestions, but not the focus of this manuscript. We have decided to emphasize the stationing behavior in the present manuscript. We have referenced our prior work which address these points for interested readers.

“Specify what you mean by "high energy dogs"; address whether the methods presented would only work with these?”

See reviewer 1 comment (now addressed on Lines 679-684).

“Specify, in min or s, how long the structural and functional runs are/or what your target minimal length is.”

We have removed the information from the manuscript and instead focused on the stationing behavior for the protocol.

“If feasible present some evidence on "how well the training worked", e.g. average movement across sessions, number of training sessions needed on average, their range, and S.D.? Basically any kind of statistics that help to evaluate how long the individual training phases require, and what the outcomes are in terms of image quality.”

Thank you for the suggestion. We have added information regarding training and testing sessions in Table 2 and Figure 3. We have conducted analyses showing that the criterion for max duration of stationing behavior was stable and at criterion before transfer testing began and that the stationing behavior immediately transferred to novel locations. We did not present image quality data because we were unable to make meaningful comparisons because of the differences in types of sequences conducted across dogs and hence focused on stationing behavior in the scanner.

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